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Molecular analysis of strains from tuberculosis patients in Polish prisons in 2004–2008. Initial analysis of the project

Molekularne dochodzenia epidemiologiczne wśród polskich więźniów chorych na gruźlicę w latach 2004–2008. Badania wstępne

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Abstract

Introduction: Correctional facilities are recognised "breeding ground" for infectious diseases. As The World Health Organization reported, the incidence of infectious diseases in prison's population is 10–100 times higher than in general population. The incidence of tuberculosis among correctional inmates in Poland in 2008 was 270/100000, that is around 10 times higher than among non-prisoners.

Materials and methods: The study included 57 *M. tuberculosis* isolates from patients in Polish prisons in 2004–2008 (5% of all diagnosed TB patient in Polish prisons 2004–2008). Primary isolation was performed with Löwenstein-Jensen (L-J) medium, species identification was done with the niacin test and gene probes test. Bacterial DNA was extracted from the L-J medium slants with the cetyltrimethylammonium bromide (CTAB) method. *Mycobacterium tuberculosis* strains were analyzed with two methods: screening for epidemiological discrimination of *M. tuberculosis* — spoligotyping and high-throughput — MIRU/VNTR.

Results: Isolates that are grouped in clusters (33 isolates) were analyzed by means of MIRU/VNTRs. In MIRU/VNTRs all strains showed different genetic patterns. Most isolates of the prisoners were grouped into two clusters: T1 53 and H3 50.

Conclusions:

1. MIRU/VNTR is a high-throughput method.
2. MIRU/VNTR is a promising method to diagnose TB transmission in Polish jails.
3. To identify the probable source of transmission, molecular analysis of strains from patients of the general population is needed.

Key words: tuberculosis, prisoners, transmission, spoligotyping, MIRU/VNTR

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Introduction

A prison environment causes serious health problems in many countries. There are more than 10 million people imprisoned worldwide. It is estimated that there are 145 prisoners per 100,000 population. The highest rates are noted in the USA — 756 per 100,000 people [1]. According to the World Health Organization (WHO), the incidence of infectious diseases is 10–100 times higher among prisoners than in the general population. That applies to active tuberculosis (TB), including multidrug-resistant TB, as well as to human immunodeficiency virus (HIV) infections [2].

It is difficult to compare tuberculosis incidence rates among prisoners and the general popula-

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tion because data on ill prisoners are not freely available. In many countries data of that kind are recorded sporadically or only in selected penal institutions. Many studies have shown that TB incidence rates are higher among the population of prisoners [3]. Analysis performed in countries of the European Area of the WHO in 2002 showed that the mean incidence rate for tuberculosis among prisoners was 232 new cases per 100,000 and was 80 times higher than in the general population. Twenty-two countries were included in that study. The highest rates were recorded in Kazakhstan — 17,808.2 per 100,000 and in Azerbaijan — 4000 per 100,000. The lowest rates (0 per 100,000) were in the Czech Republic, the Netherlands, and Portugal [4]. In a study conducted in 13 Western European countries in 2003 the incidence rate was 90/100,000 [1]. In the USA in 2003 the incidence rate was 29.4/100,000 among prisoners and 6.7/100,000 in the general population [1]. In Poland in the same year these rates were 238.7/100,000 and 26.5/100,000, respectively. A total of 408 cases of tuberculosis were recorded among Polish prisoners in 2003. Most of the cases (93%) were detected at the time of admission to the penal institution; only 7% of cases were diagnosed while serving a sentence [5].

There were 236 prisoners among the newly diagnosed cases in Poland in 2008; they accounted for 2.7% of all registered cases in the country [6]. The incidence of tuberculosis in that group of patients was around 270 cases per 100,000, giving a rate 10 times higher than in the general population [7].

A similar tuberculosis incidence rate of 260/100,000 was recorded in 2010. Two hundred and fifteen patients staying in custody and penal institutions were registered [8].

The range of health problems regarding prisoners is wide. This is the result of many factors, such as the prisoners' bad social situation and background, and too many inmates sharing one cell. All of these are related to higher incidence of tuberculosis [9].

Additional factors are alcohol and other substance addictions as well as comorbidities such as hepatitis or HIV infections [10].

Only a quick and effective diagnostic process can prevent and limit transmission of infectious diseases (including tuberculosis) in such a group. Additional problems in tracking modes of tuberculosis transmission in penal institutions are related to the fact that there are also immigrants staying in Polish prisons. In this situation it is difficult to trace the prisoner's contacts before his/her admission to a prison as well as after their release [9].

The aim of the preliminary molecular investigation conducted by the authors of this study was to determine whether and to what degree the phenomenon of tuberculosis transmission is present in Polish prisons.

Material and methods

The material for the study consisted of 57 *M. tuberculosis* isolates from patients with tuberculosis confirmed by positive cultures, staying in Polish prisons. Those patients were from the following provinces: Masovian, Łódź, Lublin, Kuyavian-Pomeranian, West-Pomeranian, Świętokrzyskie, Lesser Poland, Warmian-Masurian, Lower Silesian, and Silesian. They were all diagnosed whilst staying in custody in Polish prisons in the years 2004–2008. The studied group of 57 patients (5% of all bacteriologically confirmed tuberculosis patients in prisons in the years 2004-2008) were treated in three tuberculosis departments of prison hospitals in Potulice, Łódź, and Gdańsk.

Species identification was done with the niacin test and gene probes test (AccuProbe; GenProbe, San Diego, CA) [11].

Bacterial genomic DNA was extracted from the Löwenstein-Jensen (L-J) medium slants using the cetyltrimethylammonium bromide (CTAB) method. Isolated DNA was analysed with spoligotyping and *mycobacterial interspersed repetitive-unit-variable-number tandem repeat* (MIRU/VNTR) [11].

Spoligotyping

This is a standard method for screening in tuberculosis epidemiological investigation. The spoligotyping test was performed with use of a commercial kit (ISOGEN Bioscence BV), according to the manufacturer's instructions (fig.1).

The obtained hybridization patterns were compared to the international spoligotyping database (SpolDB4) (www.pasteur-guadeloupe.fr/tb/spoldb4). Strains having the same molecular patterns were grouped into clusters. Single hybridization patterns were defined as unique [11].

MIRU/VNTR

Subsequently, the MIRU/VNTR method was applied. It is based on the identification of 15 of the most polymorphic microsatellite sequences (55–72 nucleotides in length) in the *M. tuberculosis* genome [12]. Fifteen reactions of amplification were conducted with the use of a starter tandem complementary to regions next to MIRU sequences. PCR products were analysed on a 2% agarose gel and compared to the pattern. The results are

Figure 1. Spoligotyping pattern

presented as a set of digits that reflect the number of MIRU sequence repeats — the so-called MIRU code [13].

Results

Fifty-seven strains of *Mycobacterium tuberculosis* isolated from patients staying in penal institutions were analysed. Among those strains 31 genetic profiles were identified by spoligotyping; from those, 11 were not registered in the international database SpoIDB4.

Molecular patterns of *M. tuberculosis* were divided into two groups. The first one included 24 (42%) non-clusterable strains with unique genetic patterns. The other 33 (57.9%) strains belonged to 7 registered in the database of molecular families that have 2-13 strains in a cluster, and 3 strains having the same spoligotype not yet registered in the SpoIDB4 database (Table 1).

The strains that were identified as belonging to molecular families on the basis of spoligotyping were further analysed with a method capable of greater differentiation — MIRU/VNTR. Those 33 clusterable strains were as follows: T1 53 (22,8%), H3 50 (8,7%), T1 278 (5,3%), H1 47(5,3%), T1 612 (3,5%), T3 37 (3,5%), H3 511(3,5%), and 177777677760771 (5,3%).

Table 1. Results of spoligotyping analysis of strains from polish prisoners

Spoligotyping	Number of patients	% of patients
T1 53	13	22.8
H3 50	5	8.7
T 1278	3	5.3
H1 47	3	5.3
T1 612	2	3.5
T3 37	2	3.5
H3 511	2	3.5
177777677760	3	5.3

MIRU/VNTR showed a different 15-digit genetic code for each of the 33 strains (table 2). The obtained results did not prove the existence of tuberculosis transmission among the studied group of prisoners; however, they may be useful in future analyses.

Discussion

Our molecular analysis included *M. tuberculosis* strains obtained form 57 patients staying in Polish penal institutions and treated in three tuberculosis hospital units in the years 2004-2008. It did not show any genetic affinity in the studied group. Each of the strains had a different molecular pattern by means of MIRU/VNTR method. This preliminary investigation excludes with a high probability a common source of infection in those 57 prisoners. Rasolofo-Razanamparany et al. assessed the likelihood of tuberculosis transmission in a prison by comparison of genetic patterns of *M. tuberculosis* obtained from imprisoned patients and not imprisoned patients. The molecular test included strains isolated from 146 imprisoned persons and from 260 not imprisoned persons from Antananarivo (Madagascar) in 1994–1995. The percentage of newly diagnosed cases was significantly higher among the inmates (58.9%) than in the general population (40%), which the authors interpreted as proof that transmission was greater in the correctional setting [14]. In Poland, all prisoners undergo detailed examination at the time of admission to a penal institution, and subsequently they have a chest X-ray performed every two years [5]. It allows for quick isolation of infected individuals and prevents disease transmission. This policy is in agreement with European Prison Rules. However, this procedure allows for detection of only active forms of tuberculosis. In some countries, such as in a penal centre in Barcelona in

Table 2. Results of MIRU/VNTR analysis of strains from Polish prisoners

N.b. strains	MIRU pattern														
1	3	2	4	4	2	3	3	3	5	2	4	4	2	2	1
2	4	3	3	4	2	3	3	3	5	2	4	5	1	2	4
3	4	3	4	4	2	3	2	3	5	2	2	4	2	2	2
4	2	3	7	4	2	3	4	3	5	2	2	4	2	2	1
5	4	3	10	3	2	2	3	3	5	2	2	5	2	2	2
6	3	3	4	4	2	3	3	3	4	2	2	5	2	3	2
7	3	3	10	4	3	3	3	3	5	2	2	5	0	2	1
8	3	3	3	4	2	2	3	3	5	2	2	5	2	2	1
9	4	3	4	4	3	3	3	3	1	1	3	5	0	2	1
10	4	4	4	2	3	2	3	2	5	2	3	5	2	2	0
11	3	3	3	4	2	3	4	3	5	2	4	5	2	2	2
12	1	3	4	4	2	3	3	3	5	2	4	5	2	2	0
13	1	3	10	4	2	2	3	3	5	2	4	5	2	2	3
14	4	3	4	4	2	2	3	3	5	2	2	5	2	3	3
15	4	3	4	4	2	2	3	3	4	2	2	5	2	2	2
16	2	3	4	4	2	3	3	3	5	2	4	4	2	2	1
17	3	2	4	5	3	4	4	2	4	2	3	5	2	2	1
18	2	2	5	5	3	3	4	3	3	2	3	4	2	2	0
19	2	2	5	5	3	4	3	2	4	2	3	5	2	2	1
20	2	2	5	3	3	3	5	3	6	4	3	5	2	3	2
21	5	2	4	3	3	3	4	3	9	4	3	5	2	3	4
22	3	2	4	3	3	3	5	3	7	4	3	5	2	3	4
23	3	3	4	3	3	3	5	3	3	4	3	5	2	3	2
24	2	3	4	3	3	3	4	3	7	4	3	5	2	3	0
25	3	3	4	3	3	3	4	3	5	4	3	5	2	2	1
26	2	3	5	3	3	3	4	3	5	4	3	5	0	2	2
27	2	3	5	3	3	3	4	3	6	4	0	5	2	3	3
28	3	3	10	3	3	3	5	3	5	4	3	5	0	2	2
29	2	0	10	3	3	3	5	3	3	4	3	5	2	3	3
30	3	0	4	3	3	3	5	3	0	4	0	5	2	3	3
31	1	2	4	3	2	1	0	1	3	2	4	5	5	4	1
32	4	3	0	2	1	2	1	0	0	3	5	2	4	4	4
33	2	2	2	1	1	1	3	4	5	0	5	5	4	3	2

Objaśnienia skrótów w tekście

Spain, programmes to allow the detection of both the infection and active tuberculosis in individuals with no history of contact with tuberculosis, at the time of admission, have been developed [15].

Due to increased immigration from former Soviet Union countries in recent years, cases of tuberculosis in prisoners originating from such regions were recorded in a Polish registry. This may cause an increase in tuberculosis incidence rates in Polish prisons in the future.

The frequency of multiple drug resistance (MDR) tuberculosis in the correctional setting in former Soviet Union countries is 24.6% among newly diagnosed cases and 92.1% among those with a previous history of treatment [3]. Taking into consideration the factors presented above, a proper tuberculosis prevention and surveillance programme in penal institutions is very important.

Further detailed molecular analysis aimed at the determination of potential sources of transmission among tuberculosis patients in Polish penal institutions will be undertaken. It will compare prisoner populations with general populations in various regions of Poland.

Conclusions

Molecular analysis of strains isolated from 57 tuberculosis patients from correctional institutions showed a high usefulness of the MIRU/VNTR method.

Conflict of interest

The authors have no conflict of interest to report.

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